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(54) Title: COMPOSITIONS AND METHODS OF USE OF LDL-LIKE RECEPTOR LIGANDS FOR THE TREATMENT OF CANCER AND ANGIOGENIC-BASED DISEASE		
(57) Abstract <p>Compositions and methods effective in inhibiting abnormal or undesirable cell proliferation, particularly endothelial cell proliferation and angiogenesis related to neovascularization and tumor growth are provided. The compositions comprise naturally occurring or synthetic protein, peptide, or protein fragment capable of binding to low density lipoprotein (or low density lipoprotein-like) receptors. The compositions may be administered using a pharmaceutically acceptable carrier. The methods involve administering to a human or animal the compositions described herein in a dosage sufficient to inhibit cell proliferation, particularly endothelial cell proliferation. The methods are useful for treating diseases and processes, such as cancer, mediated by undesired and uncontrolled cell proliferation particularly by inhibiting angiogenesis. Administration of the compositions of the present invention to a human or animal having prevascularized metastasized tumors is useful for preventing the growth or expansion of such tumors.</p>		

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5 **COMPOSITIONS AND METHODS OF USE OF**
 LDL-LIKE RECEPTOR LIGANDS FOR THE TREATMENT OF
 CANCER AND ANGIOGENIC-BASED DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

10 This application claims priority to Unites States Patent
Application Serial No. 09/270,982, filed March 17, 1999.

FIELD OF THE INVENTION

15 The present invention relates to compositions and methods
for the inhibition of cellular proliferation. More particularly, the present
invention relates to the use of proteins or peptides, and/or fragments
thereof, that bind to lipoprotein receptors, particularly low density
lipoprotein receptors, and inhibit angiogenesis and angiogenesis-related
diseases.

20 **BACKGROUND OF THE INVENTION**

Cellular proliferation is a normal ongoing process in all
living organisms and is one that involves numerous factors and signals that
are delicately balanced to maintain regular cellular cycles. The general
process of cell division is one that consists of two sequential processes:
25 nuclear division (mitosis), and cytoplasmic division (cytokinesis). Because
organisms are continually growing and replacing cells, cellular proliferation
is a central process that is vital to the normal functioning of almost all
biological processes. Whether or not mammalian cells will grow and
divide is determined by a variety of feedback control mechanisms, which
30 include the availability of space in which a cell can grow, and the secretion
of specific stimulatory and inhibitory factors in the immediate environment.

When normal cellular proliferation is disturbed or somehow
disrupted, the results can affect an array of biological functions. Disruption
of proliferation could be due to a myriad of factors such as the absence or
35 overabundance of various signaling chemicals or presence of altered
environments. Some disorders characterized by abnormal cellular
proliferation include cancer, abnormal development of embryos, improper

formation of the corpus luteum, difficulty in wound healing as well as malfunctioning of inflammatory and immune responses.

Cancer is characterized by abnormal cellular proliferation. Cancer cells exhibit a number of properties that make them dangerous to the host, often including an ability to invade other tissues and to induce capillary ingrowth, which assures that the proliferating cancer cells have an adequate supply of blood. One of the defining features of cancer cells is that they respond abnormally to control mechanisms that regulate the division of normal cells and continue to divide in a relatively uncontrolled fashion until they kill the host.

Angiogenesis and angiogenesis related diseases are closely affected by cellular proliferation. As used herein, the term "angiogenesis" means the generation of new blood vessels into a tissue or organ. Under normal physiological conditions, humans or animals undergo angiogenesis only in very specific restricted situations. For example, angiogenesis is normally observed in wound healing, fetal and embryonal development and formation of the corpus luteum, endometrium and placenta. The term "endothelium" is defined herein as a thin layer of flat cells that lines serous cavities, lymph vessels, and blood vessels. These cells are defined herein as "endothelial cells". The term "endothelial inhibiting activity" means the capability of a molecule to inhibit angiogenesis in general. The inhibition of endothelial cell proliferation also results in an inhibition of angiogenesis.

Both controlled and uncontrolled angiogenesis are thought to proceed in a similar manner. Endothelial cells and pericytes, surrounded by a basement membrane, form capillary blood vessels. Angiogenesis begins with the erosion of the basement membrane by enzymes released by endothelial cells and leukocytes. The endothelial cells, which line the lumen of blood vessels, then protrude through the basement membrane. Angiogenic stimulants induce the endothelial cells to migrate through the eroded basement membrane. The migrating cells form a "sprout" off the parent blood vessel, where the endothelial cells undergo mitosis and proliferate. The endothelial sprouts merge with each other to form capillary loops, creating the new blood vessel.

Persistent, unregulated angiogenesis occurs in a multiplicity of disease states, tumor metastasis and abnormal growth by endothelial cells and supports the pathological damage seen in these conditions. The diverse pathological disease states in which unregulated angiogenesis is present have been grouped together as angiogenic-dependent, angiogenic-

associated, or angiogenic-related diseases. These diseases are a result of abnormal or undesirable cell proliferation, particularly endothelial cell proliferation.

5 The hypothesis that tumor growth is angiogenesis-dependent was first proposed in 1971 by Judah Folkman (*N. Engl. Jour. Med.* 285:1182 1186, 1971). In its simplest terms the hypothesis proposes that once tumor "take" has occurred, every increase in tumor cell population must be preceded by an increase in new capillaries converging on the tumor. Tumor "take" is currently understood to indicate a prevascular
10 phase of tumor growth in which a population of tumor cells occupying a few cubic millimeters volume and not exceeding a few million cells, survives on existing host microvessels. Expansion of tumor volume beyond this phase requires the induction of new capillary blood vessels. For example, pulmonary micrometastases in the early prevascular phase in
15 mice would be undetectable except by high power microscopy on histological sections. Further indirect evidence supporting the concept that tumor growth is angiogenesis dependent is found in U.S. Patent Nos. 5,639,725, 5,629,327, 5,792,845, 5,733,876, and 5,854,205, all of which are incorporated herein by reference.

20 Thus, it is clear that cellular proliferation, particularly endothelial cell proliferation, and most particularly angiogenesis, plays a major role in the metastasis of a cancer. If this abnormal or undesirable proliferation activity could be repressed, inhibited, or eliminated, then the tumor, although present, would not grow. In the disease state, prevention
25 of abnormal or undesirable cellular proliferation and angiogenesis could avert the damage caused by the invasion of the new microvascular system. Therapies directed at control of the cellular proliferative processes could lead to the abrogation or mitigation of these diseases.

30 What is needed therefore are compositions and methods which can inhibit abnormal or undesirable cellular proliferation, especially the growth of blood vessels into tumors. The compositions should be able to overcome the activity of endogenous growth factors in premetastatic tumors and prevent the formation of the capillaries in the tumors thereby inhibiting the development of disease and the growth of tumors. The
35 compositions should also be able to modulate the formation of capillaries in angiogenic processes, such as wound healing and reproduction. Finally, the compositions and methods for inhibiting cellular proliferation should preferably be non-toxic and produce few side effects.

SUMMARY OF THE INVENTION

Compositions and methods are provided that are effective in inhibiting abnormal or undesirable cell proliferation, particularly endothelial cell proliferation and angiogenesis related to neovascularization and tumor growth. The compositions comprise a naturally occurring or synthetic protein, peptide, or protein fragment containing all, or an active portion of low density lipoprotein receptor (LDLR) binding ligand, optionally combined in a pharmaceutically acceptable carrier.

Representative ligands useful for the present invention comprise proteins or peptides and fragments thereof belonging to the following categories: proteinases (i.e. tissue-type plasminogen activator (tPA)), proteinase inhibitors (i.e. serpins, TFPI), proteinase/inhibitor complexes (urokinase-type plasminogen activator/plasminogen activator inhibitor 1 (uPA/PAI), thrombin/antithrombin), lipoproteins (i.e. apolipoprotein B, apolipoprotein J or clusterin), and matrix proteins (i.e. thrombospondin). In addition, specific proteins or peptides comprising representative ligands useful for the present invention include: alpha2-macroglobulin, beta-amyloid precursor protein (APP), proteinase nexin II (PN2), proteinase nexin I (PN1), pro-uPA, antithrombin III, lipoprotein lipase, lactoferrin, PAI-1, horse leukocyte elastase inhibitor, protein C inhibitor, C1-inhibitor, alpha2-antiplasmin, alpha1-proteinase inhibitor, and alpha1-antichymotrypsin, heparin cofactor II.

Preferred LDLR ligands comprise proteins belonging to the protein family of serpins (serine protease inhibitors) those containing Kunitz or non-Kunitz domains such as tissue factor pathway inhibitor 1 (TFPI-1), tissue factor pathway inhibitor 2 (TFPI-2), antithrombin III, amyloid protein precursor (APP); beta-amyloid precursor protein, collagen VI, and bovine pancreatic trypsin inhibitor (BPTI).

Preferably, the protein, peptide or fragment thereof, contains all or an active portion of the above identified proteins. The term "active portion", as used herein, means a portion of a protein that inhibits cancer, preferably by inhibiting abnormal or undesirable cell proliferation. Also included in the present invention are homologs, peptides, or protein fragments, or combinations thereof of the above-identified proteins, that inhibit abnormal or undesirable cell proliferation. Most preferably, the protein, peptide, or protein fragment is a protein, peptide or protein fragment of an LDLR binding ligand.

Though not wishing to be bound by the following theory, it is believed that by inhibiting endothelial cell proliferation, the methods and compositions described herein are useful for inhibiting tumor growth and metastasis by blocking tumor vascularization.

5 The methods provided herein for treating diseases and processes mediated by undesired and uncontrolled cell proliferation, such as cancer, involve administering to a human or animal the composition described herein in a dosage sufficient to inhibit cell proliferation, particularly endothelial cell proliferation. The methods are especially useful
10 for treating or repressing the growth of tumors, particularly by inhibiting angiogenesis. Administration of the compositions to a human or animal having prevascularized metastasized tumors is useful for preventing the growth or expansion of such tumors.

 Accordingly, it is an object of the present invention to
15 provide methods and compositions for treating diseases and processes that are mediated by abnormal or undesirable cellular proliferation.

 It is another object of the present invention to provide methods and compositions for treating or repressing the growth of a cancer.

20 It is yet another object of the present invention to provide methods and compositions for therapy of cancer that has minimal side effects.

 It is another object of the present invention to provide methods and compositions for treating diseases and processes that are
25 mediated by angiogenesis.

 Yet another object of the present invention is to provide methods and compositions comprising the use of proteins, peptides, active fragments and homologs thereof, that inhibit cell proliferation, particularly endothelial cell proliferation.

30 Another object of the present invention is to provide methods and compositions for treating diseases and processes that are mediated by angiogenesis by administering antiangiogenic compounds comprising low density lipoprotein receptor ligands.

 It is another object of the present invention to provide
35 methods and compositions for treating diseases and processes that are mediated by angiogenesis by administering antiangiogenic compounds comprising low density lipoprotein receptor ligands, wherein the ligands

comprise proteinases, proteinase inhibitors, lipoproteins and matrix proteins.

5 It is another object of the present invention to provide methods and compositions for treating diseases and processes that are mediated by angiogenesis comprising administration of antiangiogenic compounds comprising low density lipoprotein receptor ligands wherein the ligands comprise antithrombin III and amyloid protein precursor.

10 It is a further object of the present invention to provide methods and compositions for reducing cancer and inhibiting tumor growth in a human or animal having cancer by administering antiangiogenic compounds comprising low density lipoprotein receptor ligands, wherein the ligands comprise proteinases, proteinase inhibitors, lipoproteins and matrix proteins.

15 It is still another object of the present invention to provide antiangiogenic compositions comprising low density lipoprotein receptor ligands, wherein the ligands comprise proteinases, proteinase inhibitors, lipoproteins and matrix proteins in combination with pharmaceutically acceptable carriers.

20 Yet another object of the present invention is to provide antiangiogenic compositions comprising low density lipoprotein receptor ligands, wherein the ligands comprise proteinases, proteinase inhibitors, lipoproteins and matrix proteins optionally combined with pharmaceutically acceptable carriers that may be administered intramuscularly, intravenously, transdermally, orally, or subcutaneously.

25 It is yet another object of the present invention to provide compositions and methods for treating diseases and processes that are mediated by angiogenesis including, but not limited to, hemangioma, solid tumors, blood borne tumors, leukemia, metastasis, telangiectasia, psoriasis, scleroderma, pyogenic granuloma, myocardial angiogenesis, Crohn's disease, plaque neovascularization, arteriovenous malformations, 30 corneal diseases, rubeosis, neovascular glaucoma, diabetic retinopathy, retrolental fibroplasia, arthritis, diabetic neovascularization, macular degeneration, wound healing, peptic ulcer, Helicobacter related diseases, fractures, keloids, vasculogenesis, hematopoiesis, ovulation, menstruation, 35 placentation, and cat scratch fever.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

5

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph showing the partial neutralization of TFPI activity by RAP.

10

Figure 2 is a dose response graph showing cell proliferation activity in the presence of various amounts of TFPI and RAP and is a representative of four experiments. Error bars represent standard deviation.

15

Figure 3 is a graph showing the dose-dependent inhibitory effect of clusterin on bFGF-induced endothelial cell proliferation.

DETAILED DESCRIPTION

20

The following description includes the best presently contemplated mode of carrying out the invention. This description is made for the purpose of illustrating the general principles of the inventions and should not be taken in a limiting sense. The entire text of the references mentioned herein are hereby incorporated in their entireties by reference, including U.S. Patent Application Serial No. 09/270,982, filed March 17, 1999, U.S. Patent Application Serial No. 09/130,273, filed August 6, 1998; and United States Patent No. 5,981,471, issued November 9, 1999.

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30

Compositions and methods for the treatment of diseases and processes that are mediated by, or associated with, abnormal or undesirable cellular proliferation are provided. The compositions comprise isolated naturally occurring or synthetic protein, peptide, or protein fragment, containing all or an active portion of ligands that bind low density lipoprotein (or low density lipoprotein-like) receptors (LDLR). For delivery to a human or animal, the compositions may optionally comprise a pharmaceutically acceptable carrier.

35

The term "active portion" is defined herein as the antiproliferative portion of the protein necessary for binding LDLR. The active portion has the ability to inhibit cell proliferation such as endothelial cell proliferation by *in vivo* or *in vitro* assays or other known techniques. Preferably, the LDLR ligand compositions of the present invention

comprise proteins belonging to the categories of proteinases (i.e. tissue-type plasminogen activator (tPA)), proteinase inhibitors (i.e. serpins, TFPI), proteinase/inhibitor complexes (urokinase-type plasminogen activator/plasminogen activator inhibitor 1 (uPA/PAI), thrombin/antithrombin), lipoproteins (i.e. apolipoprotein B), and matrix proteins (i.e. thrombospondin). Most preferably, the protein, peptide, or protein fragment is a protein, peptide or protein fragment comprising representative ligands including, but not limited to, alpha2-macroglobulin, beta-amyloid precursor protein (APP), proteinase nexin II (PN2), proteinase nexin I (PN1), pro-uPA, lipoprotein lipase, lactoferrin, PAI-1, horse leukocyte elastase inhibitor, protein C inhibitor, C1-inhibitor, alpha2-antiplasmin, alpha1-proteinase inhibitor, alpha1-antichymotrypsin, heparin cofactor II, and antithrombin III.

As noted above, the compositions of the present invention may be optionally combined with a pharmaceutical carrier. The term "carrier" as used herein comprises delivery mechanisms known to those skilled in the art including, but not limited to, keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) and other adjuvants. It is to be understood that the low density lipoprotein receptor ligand compositions of the present invention can further comprise adjuvants, preservatives, diluents, emulsifiers, stabilizers, and other components that are known and used for pharmaceutical compositions of the prior art. Any adjuvant system known in the art can be used for the compositions of the present invention. Such adjuvants include, but are not limited to, Freund's incomplete adjuvant, Freund's complete adjuvant, polydispersed β -(1,4) linked acetylated mannan ("Acemannan"), TITERMAX[®] (polyoxyethylene-polyoxypropylene copolymer adjuvants from CytRx Corporation (Norcross, Georgia), modified lipid adjuvants from Chiron Corporation (Emeryville, California), saponin derivative adjuvants from Aguila Biopharmaceuticals (Worcester, Massachusetts), killed *Bordetella pertussis*, the lipopolysaccharide (LPS) of gram-negative bacteria, large polymeric anions such as dextran sulfate, and inorganic gels such as alum, aluminum hydroxide, or aluminum phosphate, ovalbumin; flagellin; thyroglobulin; serum albumin of any species; gamma globulin of any species; and polymers of D- and/or L- amino acids.

Alternatively, the gene for the protein, peptide, or protein fragment, containing all or an active portion of the protein, may be delivered in a vector for continuous administration using gene therapy

techniques. The vector may be administered in a vehicle having specificity for a target site, such as a tumor.

In accordance with the methods of the present invention, the compositions described herein, containing a protein, peptide, or protein fragment including all or an active portion of an LDLR ligand, optionally combined in a pharmaceutically acceptable carrier, is administered to a human or animal exhibiting undesirable cell proliferation in an amount sufficient to inhibit the undesirable cell proliferation, particularly endothelial cell proliferation, angiogenesis or an angiogenesis-related disease, such as cancer.

Definitions

The terms "a", "an" and "the" as used herein are defined to mean one or more and include the plural unless the context is inappropriate.

The term "peptides," are chains of amino acids (typically L-amino acids) whose alpha carbons are linked through peptide bonds formed by a condensation reaction between the carboxyl group of the alpha carbon of one amino acid and the amino group of the alpha carbon of another amino acid. The terminal amino acid at one end of the chain (*i.e.*, the amino terminal) has a free amino group, while the terminal amino acid at the other end of the chain (*i.e.*, the carboxy terminal) has a free carboxyl group. As such, the term "amino terminus" (abbreviated N-terminus) refers to the free alpha-amino group on the amino acid at the amino terminal of the peptide, or to the alpha-amino group (imino group when participating in a peptide bond) of an amino acid at any other location within the peptide. Similarly, the term "carboxy terminus" (abbreviated C-terminus) refers to the free carboxyl group on the amino acid at the carboxy terminus of a peptide, or to the carboxyl group of an amino acid at any other location within the peptide.

Typically, the amino acids making up a peptide are numbered in order, starting at the amino terminal and increasing in the direction toward the carboxy terminal of the peptide. Thus, when one amino acid is said to "follow" another, that amino acid is positioned closer to the carboxy terminal of the peptide than the preceding amino acid.

The term "residue" is used herein to refer to an amino acid (D or L) that is incorporated into a peptide by an amide bond. As such, the amino acid may be a naturally occurring amino acid or, unless otherwise limited, may encompass known analogs of natural amino acids that

function in a manner similar to the naturally occurring amino acids (*i.e.*, amino acid mimetics). Moreover, an amide bond mimetic includes peptide backbone modifications well known to those skilled in the art.

5 The phrase "consisting essentially of" is used herein to exclude any elements that would substantially alter the essential properties of the peptides to which the phrase refers. Thus, the description of a peptide "consisting essentially of . . ." excludes any amino acid substitutions, additions, or deletions that would substantially alter the biological activity of that peptide.

10 Furthermore, one of skill will recognize that, as mentioned above, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are conservatively modified variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

- 15
- 1) Alanine (A), Serine (S), Threonine (T);
 - 20 2) Aspartic acid (D), Glutamic acid (E);
 - 3) Asparagine (N), Glutamine (Q);
 - 4) Arginine (R), Lysine (K);
 - 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
 - 25 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the peptides described herein do not contain materials normally associated with their *in situ* environment. Typically, the isolated, antiproliferative peptides described herein are at least about 80% pure, usually at least about 90%, and preferably at least about 95% as measured by band intensity on a silver stained gel.

30 Protein purity or homogeneity may be indicated by a number of methods well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualization upon staining. For certain purposes high resolution will be needed and HPLC or a similar means for purification utilized.

When the antiproliferative peptides are relatively short in length (*i.e.*, less than about 50 amino acids), they are often synthesized using standard chemical peptide synthesis techniques.

5 Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence is a preferred method for the chemical synthesis of the antiproliferative peptides described herein. Techniques for solid phase synthesis are known to those skilled in the art.

10 Alternatively, the antiproliferative peptides described herein are synthesized using recombinant nucleic acid methodology. Generally, this involves creating a nucleic acid sequence that encodes the peptide, placing the nucleic acid in an expression cassette under the control of a particular promoter, expressing the peptide in a host, isolating the expressed peptide or polypeptide and, if required, renaturing the peptide.
15 Techniques sufficient to guide one of skill through such procedures are found in the literature.

Once expressed, recombinant peptides can be purified according to standard procedures, including ammonium sulfate precipitation, affinity columns, column chromatography, gel
20 electrophoresis and the like. Substantially pure compositions of about 50 to 95% homogeneity are preferred, and 80 to 95% or greater homogeneity are most preferred for use as therapeutic agents.

One of skill in the art will recognize that after chemical synthesis, biological expression or purification, the antiproliferative
25 peptides may possess a conformation substantially different than the native conformations of the constituent peptides. In this case, it is often necessary to denature and reduce the antiproliferative peptide and then to cause the peptide to re-fold into the preferred conformation. Methods of reducing and denaturing proteins and inducing re-folding are well known to those of
30 skill in the art.

As employed herein, the phrase "biological activity" refers to the functionality, reactivity, and specificity of compounds that are derived from biological systems or those compounds that are reactive to them, or other compounds that mimic the functionality, reactivity, and
35 specificity of these compounds. Examples of suitable biologically active compounds include enzymes, antibodies, antigens and proteins.

The term "bodily fluid," as used herein, includes, but is not limited to, saliva, gingival secretions, cerebrospinal fluid, gastrointestinal

fluid, mucous, urogenital secretions, synovial fluid, blood, serum, plasma, urine, cystic fluid, lymph fluid, ascites, pleural effusion, interstitial fluid, intracellular fluid, ocular fluids, seminal fluid, mammary secretions, and vitreal fluid, and nasal secretions.

5

Low Density Lipoprotein Receptor Characteristics

As used herein, the term "low density lipoprotein receptor (LDLR)" includes a family of proteins comprising low density lipoprotein (LDL), low density lipoprotein receptor-related protein (LRP), very low
10 density lipoprotein receptor (VLDLR), α_2 macroglobulin receptor/low-density-lipoprotein-receptor-related protein (α_2 MR/LRP), gp330/megalin apolipoprotein E receptor 2 (apoER2) and receptor LR8B.

The LDL-receptor family of proteins is characterized by transmembrane proteins comprising approximatey four members: LDL,
15 LRP, VLDLR, and gp330/megalin (see Strickland *et al. FASEB* 9:890-898 (1995) which is incorporated herein in its entirety). These proteins exhibit similar structural patterns and perform related functions such as the mediation of endocytosis and cellular degradation of a diverse variety of ligands. One common structural aspect of the LDL-receptor family is the
20 presence, in the cytoplasmic domain, of one or more repeats of an NPXY sequence responsible for targeting the receptor to clathrin-coated pits of the cells, while the extracellular domain contains multiple copies of the epidermal growth factor-like domain responsible for the release of the receptor ligands within the endosomes.

25

Ligands Binding Low Density Lipoprotein Receptors

The novel findings of the present inventors demonstrate that the antiangiogenic, or antiproliferative, activity of compounds such as TFPI is mediated by a LDL receptor-like protein expressed on the surface of
30 endothelial cells. As shown in Example 2, the antiproliferative activity of TFPI was reduced in the presence of LDL (an LDLR ligand). In addition, further support for the role of LDLR is provided wherein the inventors demonstrated that antiproliferative activity of TFPI was reduced in the presence of VLDLR antibodies.

35

Ligands that are known to be endocytosed via the LDL receptor family of proteins comprise members of the following categories: proteinases (e.g., tPA), proteinase inhibitors (e.g., serpins, TFPI),

proteinase/inhibitor complexes (uPA/PAI, thrombin/antithrombin), lipoproteins (e.g. apolipoprotein B), and matrix proteins (e.g., thrombospondin). Preferred LDLR ligands comprise α_2 -macroglobulin, beta-amyloid precursor protein, proteinase nexin II, proteinase nexin I, pro-uPA, lipoprotein lipase, lactoferrin, PAI-1, horse leukocyte elastase inhibitor, protein C inhibitor, C1-inhibitor, α_2 -antiplasmin, alpha1-proteinase inhibitor, alpha1-antichymotrypsin, heparin cofactor II, tissue-type plasminogen activator, antithrombin III, tissue factor pathway inhibitor, apolipoprotein B, apolipoprotein J, clusterin, thrombospondin, and active fragments thereof.

In particular, ligands belonging to the serpin superfamily (serine proteinase inhibitors) having either Kunitz or non-Kunitz domains are preferred for the methods and compositions of the present invention. Serpins are a family of structurally similar proteins known to irreversibly inhibit a wide range of serine proteases. Their tertiary structure consists of 3 beta sheets (named A, B, and C), several α -helices (designated A through I), and a flexible reactive loop that contains highly variable residues. The reactive site is located in the reactive loop, approximately 30-40 amino acids from the carboxy-terminal region of the protein, and includes a peptide bond that mimics the normal substrate of the target protease. Upon binding to a protease, serpins undergo a profound conformational change in which the active loop becomes inserted into the beta sheet A. Serpin family ligands comprise tissue factor pathway inhibitor 1 (TFPI-1) and tissue factor pathway inhibitor 2 (TFPI-2), antithrombin III, amyloid protein precursor (APP); amyloid beta precursor protein, collagen VI, bovine pancreatic trypsin inhibitor (BPTI), α_1 -proteinase inhibitor, α_1 -antichymotrypsin, α_2 -antiplasmin, heparin cofactor II, protein C inhibitor (PCI), proteinase nexin-1 (PN-1), and the plasminogen activator inhibitors (PAI-1 and PAI-2).

One protein representative of protease inhibitors that bind LDLR is TFPI. TFPI is a glycoprotein having a molecular weight of approximately 32 to 45 kilodaltons. TFPI is composed of approximately 276 amino acids organized in a structure that includes an acidic amino terminus followed by three Kunitz-type protease inhibitor domains,

referred to as Kunitz-1, Kunitz-2, and Kunitz-3, and a basic carboxyl terminal region.

TFPI, also known to those skilled in the art as lipoprotein-associated coagulation inhibitor, is a protease inhibitor that plays an important role in the regulation of tissue factor-induced blood coagulation. Generally, TFPI is found in plasma, in platelets, and on endothelium. Typically, at a site of blood vessel injury after the bleeding has stopped, the concentration of TFPI is three times higher than the normal levels found in plasma. Intravascular TFPI exists in several forms, which are known to those skilled in the art. The predominant forms of plasma TFPI have molecular weights of 34 and 41 KDa but other forms with higher molecular weights are also present. TFPI is synthesized in endothelial cells and is exocytosed toward the surface of the cells where it remains bound to heparin sulfate proteoglycans (HSPGs) (see Narita *et al.* *Journ. Biol. Chemistry* 270(42):24800-24804 (1995)).

Other proteins that contain Kunitz type domains and may be used in the compositions of the present invention comprise amyloid precursor protein (APP), collagen VI and bovine pancreatic trypsin inhibitor (BPTI). APP contains Kunitz protease inhibitor domains and exists in at least four different forms that differ in the number of amino acids ranging from approximately 700-800 residues. (see Niwano *et al.* *J. Lab. Clin. Med.*, 125(2):215-256 (1995)). Collagen VI is found in blood vessel walls, has a triple-helical structure and contains a Kunitz type protease inhibitor domain. (see Kehrel, *Semin Thromb. Hemost.* 21(2):123-9 (1995)). Bovine pancreatic trypsin inhibitor (BPTI) is a 58-residue protein with three disulfide bonds that belongs to the Kunitz family of serine proteinase inhibitors. (see Moss *et al.* *J. Gen. Physiol.* 108(6): 473-84 (1996)).

In an alternative embodiment, a preferred category of LDLR binding ligands comprises ligand complexes. Whereas ligand complexes are internalized by LDL receptor-like proteins, the individual components of the complexes are typically not internalized by themselves. For example, neither plasminogen activator inhibitor 1 (PAI-1), nor urokinase-type plasminogen activator (uPA) individually bind to LRP. Upon complexing with each other however, PAI-1 undergoes a conformational change that allows the uPA/PAI-1 complex to bind to LRP via the PAI-1 component. Such ligand complexes comprise for example protease/serpin complexes such as thrombin/antithrombin or uPA/antithrombin. Additional

ligand complexes included, but limited within the scope of the present invention comprise uPA/PAI-1, tPA/PAI-1, uPA/PN-1 (proteinase nexin-1), elastase/ α_1 proteinase inhibitor, trypsin/ α_1 -proteinase inhibitor, thrombin/heparin cofactor II and thrombin PAI-1, uPA/PCI (protein C inhibitor), uPA/antithrombin III, uPA/C1-inhibitor, low molecular mass uPA/PAI-1, low molecular mass uPA/PCI, tPA/PCI, thrombin/PCI, thrombin/PN-1. Serine proteinase/serpin complexes are described for example by Kasza et al. Eur. J. Biochem. 248:270-281 (1997).

The LDLR ligands of the present invention may be isolated from body fluids including, but not limited to, serum, urine, and ascites, or may be synthesized by chemical or biological methods, such as cell culture, recombinant gene expression, and peptide synthesis. Recombinant techniques include gene amplification from DNA sources using the polymerase chain reaction (PCR), and gene amplification from RNA sources using reverse transcriptase/PCR. LDLR ligands are extracted from body fluids by known protein extraction methods, particularly the method described by Novotny, W.F., et al., J. Biol. Chem. 264:18832-18837 (1989).

Peptides or Protein Fragments

Peptides or protein fragments comprising LDLR binding ligands can be produced from the proteins described above and tested for antiproliferative or antiangiogenic activity using techniques and methods known to those skilled in the art. For example, full length recombinant TFPI (rTFPI) can be produced using the Baculovirus gene expression system. Full length proteins can be cleaved into individual domains or digested using various methods such as, for example, the method described by Enjyoji et al. (Biochemistry 34:5725-5735 (1995)). In accordance with the method of Enjyoji et al., rTFPI is treated with a digestion enzyme, human neutrophil elastase, and the digest purified using a heparin column. Human neutrophil elastase cleaves TFPI at Leu⁸⁹ into two fragments: one containing Kunitz-1 and the other containing Kunitz-2 and Kunitz-3. To produce additional fragments, the fragment containing Kunitz-2 and Kunitz-3 (Kunitz-2/Kunitz-3) is preferably treated with a digestion compound, hydroxylamine, according to the method of Balian et al. (Biochemistry 11:3798-3806 (1972)), and the digest purified using a heparin column. Hydroxylamine cleaves the fragment containing Kunitz-2

and Kunitz-3 into two fragments: one containing Kunitz-3 and the other containing the Kunitz-2 domain.

Alternatively, fragments are prepared by digesting the entire protein, or large fragments thereof exhibiting anti-proliferative activity, to remove one amino acid at a time. Each progressively shorter fragment is then tested for anti-proliferative activity. Similarly, fragments of various lengths may be synthesized and tested for anti-proliferative activity. By increasing or decreasing the length of a fragment, one skilled in the art may determine the exact number, identity, and sequence of amino acids within the protein that are required for anti-proliferative activity using routine digestion, synthesis, and screening procedures known to those skilled in the art.

Anti-proliferative activity is evaluated *in situ* by testing the ability of the fragments to inhibit the proliferation of new blood vessel cells, referred to herein as the inhibition of angiogenesis. A suitable assay is the chick embryo chorioallantoic membrane (CAM) assay described by Crum *et al.*, *Science* 230:1375 (1985) and described in U.S. Patent No. 5,001,116. The CAM assay is briefly described as follows. Fertilized chick embryos are removed from their shell on day 3 or 4, and a methylcellulose disc containing the fragment of interest is implanted on the chorioallantoic membrane. The embryos are examined 48 hours later and, if a clear avascular zone appears around the methylcellulose disc, the diameter of that zone is measured. The larger the diameter of the zone, the greater the anti-angiogenic activity. Another suitable assay is the HUVEC assay as described in Example 2.

The active fragment is preferably a fragment containing that portion of the ligand that is necessary for binding LDLR. In particular, ligands belonging to the family of protease inhibitors, having either Kunitz or non-Kunitz domains, are preferred. Serpin ligands, include but are not limited to, tissue factor pathway inhibitor 1 (TFPI-1), tissue factor pathway inhibitor 2 (TFPI-2), antithrombin III, amyloid protein precursor (APP), amyloid beta precursor protein, collagen VI, bovine pancreatic trypsin inhibitor (BPTI), α_1 -proteinase inhibitor, alpha1-antichymotrypsin, alpha2-antiplasmin, heparin cofactor II, protein C inhibitor (PCI), proteinase nexin-1 (PN-1), and the plasminogen activator inhibitors (PAI-1 and PAI-2).

As discussed above, one of skill in the art will recognize that, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are conservatively modified variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Accordingly, also included in the present invention are peptides having conservatively modified variations in comparison to the claimed peptides, wherein the chemical reactivity of the peptide is not significantly different from that of the claimed peptide.

Formulations

The naturally occurring or synthetic protein, peptide, or protein fragment, containing all or an active portion of an LDLR ligand can be prepared in a physiologically acceptable formulation, such as in a pharmaceutically acceptable carrier, using known techniques. For example, the protein, peptide or protein fragment is combined with a pharmaceutically acceptable excipient to form a therapeutic composition.

Alternatively, the gene for the protein, peptide, or protein fragment, containing all or an active portion of an LDLR ligand, is delivered in a vector for continuous administration using gene therapy techniques. The vector may be administered in a vehicle having specificity for a target site, such as a tumor.

The composition may be in the form of a solid, liquid or aerosol. Examples of solid compositions include pills, creams, and implantable dosage units. Pills may be administered orally. Therapeutic creams may be administered topically. Implantable dosage units may be administered locally, for example, at a tumor site, or may be implanted for systematic release of the therapeutic composition, for example, subcutaneously. Examples of liquid compositions include formulations adapted for injection subcutaneously, intravenously, intra-arterially, and formulations for topical and intraocular administration. Examples of aerosol formulations include inhaler formulations for administration to the lungs.

The composition may be administered by standard routes of administration. In general, the composition may be administered by topical, oral, rectal, nasal or parenteral (for example, intravenous,

subcutaneous, or intermuscular) routes. In addition, the composition may be incorporated into sustained release matrices such as biodegradable polymers, the polymers being implanted in the vicinity of where delivery is desired, for example, at the site of a tumor. The method includes
5 administration of a single dose, administration of repeated doses at predetermined time intervals, and sustained administration for a predetermined period of time.

A sustained release matrix, as used herein, is a matrix made of materials, usually polymers which are degradable by enzymatic or
10 acid/base hydrolysis or by dissolution. Once inserted into the body, the matrix is acted upon by enzymes and body fluids. The sustained release matrix desirably is chosen by biocompatible materials such as liposomes, polylactides (polylactide acid), polyglycolide (polymer of glycolic acid), polylactide co-glycolide (copolymers of lactic acid and glycolic acid),
15 polyanhydrides, poly(ortho)esters, polypeptides, hyaluronic acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids, phospholipids, polysaccharides, nucleic acids, polyamino acids, amino acids such as phenylalanine, tyrosine, isoleucine, polynucleotides, polyvinyl propylene, polyvinylpyrrolidone and silicone. A preferred biodegradable matrix is a
20 matrix of one of either polylactide, polyglycolide, or polylactide co-glycolide (co-polymers of lactic acid and glycolic acid).

The dosage of the composition will depend on the condition being treated, the particular composition used, and other clinical factors such as weight and condition of the patient, and the route of administration.

25 Further, the term "effective amount" refers to the amount of the composition which, when administered to a human or animal, inhibits undesirable cell proliferation, particularly endothelial cell proliferation, causing a reduction in cancer or inhibition in the spread and proliferation of cancer. The effective amount is readily determined by one of skill in the art
30 following routine procedures.

For example, antiproliferative compositions of the present invention may be administered parenterally or orally in a range of approximately 1.0 μ g to 1.0 mg per patient, though this range is not intended to be limiting. The actual amount of antiproliferative composition
35 required to elicit an appropriate response will vary for each individual patient depending on the potency of the composition administered, the condition being treated and on the response of the individual. Consequently, the specific amount administered to an individual will be

determined by routine experimentation and based upon the training and experience of one skilled in the art.

The composition may be administered in combination with other compositions and procedures for the treatment of diseases. For example, unwanted cell proliferation may be treated conventionally with surgery, radiation or chemotherapy in combination with the administration of the composition, and additional doses of the composition may be subsequently administered to the patient to stabilize and inhibit the growth of any residual unwanted cell proliferation.

LDL Receptor Ligand Antibodies

The present invention further comprises LDLR ligand antibodies that may be used for diagnostic as well as therapeutic purposes. The antibodies provided herein are monoclonal or polyclonal antibodies having binding specificity for LDLR ligands. The preferred antibodies are monoclonal antibodies, due to their higher specificity for the ligands. The antibodies exhibit minimal or no crossreactivity with other proteins or peptides. Preferably, the antibodies are specific for ligands comprising proteinases, proteinase inhibitors, serpins, proteinase/inhibitor complexes, thrombin/antithrombin, lipoproteins, and matrix proteins.

Monoclonal antibodies are prepared by immunizing an animal, such as a mouse or rabbit, with a whole or immunogenic portion of an LDLR ligand, such as antithrombin III. Spleen cells are harvested from the immunized animals and hybridomas generated by fusing sensitized spleen cells with a myeloma cell line, such as murine SP2/O myeloma cells (ATCC, Manassas, VA). The cells are induced to fuse by the addition of polyethylene glycol. Hybridomas are chemically selected by plating the cells in a selection medium containing hypoxanthine, aminopterin and thymidine (HAT).

Hybridomas are subsequently screened for the ability to produce monoclonal antibodies against LDLR ligands. Hybridomas producing antibodies that bind to the LDLR ligands are cloned, expanded and stored frozen for future production. The preferred hybridoma produces a monoclonal antibody having the IgG isotype, more preferably the IgG1 isotype.

The polyclonal antibodies are prepared by immunizing animals, such as mice or rabbits with LDLR ligands as described above. Blood sera is subsequently collected from the animals, and antibodies in the

sera screened for binding reactivity against the LDLR ligands, preferably the antigens that are reactive with the monoclonal antibody described above.

5 Either the monoclonal antibodies or the polyclonal antibodies, or both may be labeled directly with a detectable label for identification and quantitation of LDLR ligands in a biological or environmental sample as described below. Labels for use in immunoassays are generally known to those skilled in the art and include enzymes, radioisotopes, and fluorescent, luminescent and chromogenic
10 substances including colored particles, such as colloidal gold and latex beads. The antibodies may also be bound to a solid phase to facilitate separation of antibody-antigen complexes from non-reacted components in an immunoassay. Exemplary solid phase substances include, but are not limited to, microtiter plates, test tubes, magnetic, plastic or glass beads and
15 slides. Methods for coupling antibodies to solid phases are well known to those skilled in the art.

 Alternatively, the antibodies may be labeled indirectly by reaction with labeled substances that have an affinity for immunoglobulin, such as protein A or G or second antibodies. The antibodies may be
20 conjugated with a second substance and detected with a labeled third substance having an affinity for the second substance conjugated to the antibody. For example, the antibodies may be conjugated to biotin and the antibody-biotin conjugate detected using labeled avidin or streptavidin. Similarly, the antibodies may be conjugated to a hapten and the antibody-hapten conjugate detected using labeled anti-hapten antibody. These and
25 other methods of labeling antibodies and assay conjugates are well known to those skilled in the art.

 Sensitive immunoassays employing one or more of the antibodies described above are provided by the present invention. The
30 immunoassays are useful for detecting the presence or amount of LDLR ligands in a variety of samples, particularly biological samples, such as human or animal biological fluids or. The samples may be obtained from any source in which the LDLR ligands may exist. For example, the sample may include, but is not limited to, blood, saliva, semen, tears, and urine.

35 The antibody-antigen complexes formed in the immunoassays of the present invention are detected using immunoassay methods known to those skilled in the art, including sandwich immunoassays and competitive immunoassays. The antibody-antigen

complexes are exposed to antibodies similar to those used to capture the antigen, but which have been labeled with a detectable label. Suitable labels include: chemiluminescent labels, such as horseradish peroxidase; electrochemiluminescent labels, such as ruthenium and aequorin; bioluminescent labels, such as luciferase; fluorescent labels such as FITC; and enzymatic labels such as alkaline phosphatase, β -galactosidase, and horseradish peroxidase.

The labeled complex is then detected using a detection technique or instrument specific for detection of the label employed. Soluble antigen or antigens may also be incubated with magnetic beads coated with non-specific antibodies in an identical assay format to determine the background values of samples analyzed in the assay.

Diseases and Conditions to be Treated

The methods and compositions described herein are useful for treating human and animal diseases and processes mediated by abnormal or undesirable cellular proliferation, particularly abnormal or undesirable endothelial cell proliferation, including, but not limited to, hemangioma, solid tumors, leukemia, metastasis, telangiectasia psoriasis scleroderma, pyogenic granuloma, myocardial angiogenesis, plaque neovascularization, coronary collaterals, ischemic limb angiogenesis, corneal diseases, rubeosis, neovascular glaucoma, diabetic retinopathy, retrolental fibroplasia, arthritis, diabetic neovascularization, macular degeneration, wound healing, peptic ulcer, fractures, keloids, vasculogenesis, hematopoiesis, ovulation, menstruation, and placentation. The method and composition are particularly useful for treating angiogenesis-related disorders and diseases by inhibiting angiogenesis.

The methods and compositions described herein are particularly useful for treating cancer, arthritis, macular degeneration, and diabetic retinopathy. Administration of the compositions to a human or animal having prevascularized metastasized tumors is useful for preventing the growth or expansion of such tumors.

The compositions and methods are further illustrated by the following non-limiting examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing

from the spirit of the present invention and/or the scope of the appended claims.

EXAMPLE 1

5 *Human Umbilical Vascular Endothelial Cell Assay*

Human umbilical vascular endothelial cells (HUVECs) and their media (EGM and EBM) were purchased from Clonetics (San Diego, CA).

10 HUVECs were routinely cultured to confluency in EGM. The cells were trypsinized and plated in a 96-well plate at 5,000 cells per 100 μ l EBM supplemented with 2% serum and antibiotics. The cells were allowed to adhere to the plate for at least 2 hrs. Then, bFGF at 10 ng/ml and various concentrations of an antiangiogenic agent were added to the wells. The cells were cultured for 48 hrs at 37°C in a 5% CO₂ atmosphere. Cell proliferation was determined using a uridine incorporation method (Boeringer Mannheim Corporation, Indianapolis, IN).

EXAMPLE 2

20 *Endothelial Cell Proliferation Assay in the Presence of TFPI and LDL*

Proliferation assays familiar to those skilled in the art using human umbilical vein endothelial cells HUVECs were conducted using tissue factor pathway inhibitor (TFPI) and low density lipoproteins (LDL). (LDL is a LDLR ligand known to induce growth of endothelial cells).

25 *Materials and Methods*

The materials for this experiment included HUVECs and media for their proliferation, Endothelial Cell Basal Medium (EBM) and Endothelial Cell Growth Medium (EGM), (Clonetics, San Diego, CA). Also used was full length TFPI (American Diagnostica Inc., Greenwich, CT). In addition, a cell proliferation ELISA BrdU (Boehringer Mannheim Corporation, Indianapolis, IN), bFGF (R&D, Minneapolis, MN) and LDL (PerImmune, Inc. Rockville MD).

35 The proliferation assay involved the routine culturing of HUVECs to confluency in EGM media. The cells were trypsinized and plated in a 96-well plate at 5000 cells per well per 100 μ L EBM media. The cells were allowed to adhere to the plate for at least two hours. Next, bFGF at 10 ng/ml and full length TFPI at various concentrations was added

to the wells. The cells were cultured for 48 hours after which cell proliferation was determined using a standard uridine incorporation method.

5 *Results*

In the absence of LDL, 150 nM TFPI inhibited bFGF-induced proliferation of HUVECs by 50%. Under the same experimental conditions but in the presence of 100 µg/ml LDL, the same concentration of TFPI inhibited proliferation of HUVECs by only 30%. This partial
10 reduction in activity of TFPI demonstrates that a LDLR-like protein mediates the antiproliferative effect of TFPI. Though not wishing to be bound by the following theory, engagement of the receptor by LDL results in partial internalization of the receptor which prevents its ligation with TFPI and the induction of an antimitotic signal.

15

EXAMPLE 3

*Endothelial Cell Proliferation Assay in the Presence of
TFPI and VLDLR Antibodies*

Previous experiments conducted by the inventors demonstrated that
20 550 nM TFPI inhibited bFGF-induced proliferation of HUVECs by 90%. When HUVECs were pre-incubated in the presence of 1µM VLDLR antibodies (American Red Cross (Rockville, MD)) for an hour at 37°C, the same concentration of TFPI inhibited bFGF-induced proliferation of HUVECs by 50%. This partial reduction in the TFPI activity demonstrates
25 that, at least partially, VLDLR mediates the antiproliferative property of TFPI.

EXAMPLE 4

*Proliferation Assay in the Presence of
Receptor Associated Protein*

30

Proliferation assays as described in Examples 1 and 2 were conducted to assess the effect of receptor associated protein (RAP) on proliferation of endothelial cells. RAP is a 39 Kd protein that binds with high affinity to the members of the LDL receptor family of proteins and
35 antagonizes their ligand binding properties.

TFPI at 275nM concentration inhibited bFGF-induced proliferation of HUVECs by 100%. Under the same conditions and in the

presence of 100 nM RAP (American Red Cross (Rockville, MD)), the same concentration of TFPI inhibited bFGF-driven replication of HUVECs only by approximately 50%. Again, partial neutralization of TFPI's activity by RAP, demonstrates that a LDLR-like protein is involved in the transduction of TFPI's antimitotic signal. The results of this experiment are provided graphically in Figure 1.

EXAMPLE 5

Endothelial Cell Migration Assay in the Presence of TFPI and RAP

Proliferation assays as described in Examples 1 and 2 were conducted to assess the effect of RAP on proliferation of endothelial cells. RAP is a 39 Kd protein that binds with high affinity to the members of the LDL receptor family of proteins and antagonizes their ligand binding properties.

TFPI inhibits bFGF-induced migration of HUVECs in a dose-dependent manner. Presence of RAP in the culture media at 500 nM concentration neutralized TFPI's antimigratory activity. The results of this experiment are provided graphically in Figure 2.

EXAMPLE 6

Endothelial Cell Proliferation Assay in the Presence of Clusterin

Clusterin or apolipoprotein J is a 80 Kd multifunctional protein involved in the terminal complement pathway. Clusterin is synthesized by endothelial cells and internalized by gp330/megalin receptor and LRP (Zlokovic et al. Proc. Natl. Acad. Sci. USA 1996 93(9), 4229-4234, and Morales et al. 1996 Biol Reprod 55(3), 676-683).

Proliferation assays conducted as described above in Examples 1 and 2, demonstrate that clusterin inhibits bFGF-induced endothelial cell proliferation in a dose-dependent manner. Results of this experiment are provided graphically in Figure 3.

It should be understood, of course, that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

CLAIMS

We claim:

- 5 1. A method of treating a human or animal having undesirable cell proliferation comprising,
 administering to the human or animal an effective amount of a composition comprising an isolated protein or peptide, or antiproliferative fragment thereof,
10 wherein the protein or peptide is capable of binding to a low density lipoprotein receptor, and
 wherein the effective amount is sufficient to inhibit the undesirable cell proliferation.
- 15 2. The method of Claim 1, wherein the low density lipoprotein receptor comprises low density lipoprotein, low density lipoprotein receptor-related protein, very low density lipoprotein receptor, gp330/megalin and active fragments thereof.
- 20 3. The method of Claim 1, wherein isolated protein or peptide comprises a protein selected from the group consisting of proteinases, proteinase inhibitors, serpins, proteinase/inhibitor complexes, thrombin/antithrombin, lipoproteins, matrix proteins and active fragments thereof.
- 25 4. The method of Claim 1, wherein isolated protein or peptide is selected from the group consisting of alpha2-macroglobulin, beta-amyloid precursor protein, proteinase nexin I, proteinase nexin II, pro-uPA, lipoprotein lipase, lactoferrin, PAI-1, horse leukocyte elastase inhibitor, protein C inhibitor, C1-inhibitor, alpha2-antiplasmin, alpha1-proteinase inhibitor, alpha1-antichymotrypsin, heparin cofactor II, tissue-type plasminogen activator, antithrombin III, tissue factor pathway inhibitor, apolipoprotein B, apolipoprotein J, clusterin, thrombospondin, tissue factor pathway inhibitor 1, tissue factor pathway inhibitor 2, amyloid protein precursor, amyloid beta precursor protein, collagen VI, bovine pancreatic trypsin inhibitor, and active fragments thereof.
- 30
- 35

5. The method of Claim 3, wherein the proteinase/inhibitor complexes comprise urokinase-type plasminogen activator/plasminogen activator inhibitor 1 complex, or thrombin/antithrombin complex.

6. The method of Claim 1, wherein the wherein the low density lipoprotein receptor comprises low density lipoprotein receptor-related protein and wherein the isolated protein or peptide, or antiproliferative fragment thereof comprises alpha2-macroglobulin.

7. The method of Claim 1, wherein the wherein the low density lipoprotein receptor comprises low density lipoprotein receptor-related protein and wherein the isolated protein or peptide, or antiproliferative fragment thereof comprises antithrombin III.

8. The method of Claim 1, wherein the composition further comprises a pharmaceutically acceptable excipient, carrier or sustained-release matrix.

9. The method of Claim 1, wherein the undesirable cell proliferation is undesirable endothelial cell proliferation.

10. The method of Claim 9 wherein the inhibition of endothelial cell proliferation inhibits neovascularization.

11. The method of Claim 1, wherein the undesirable cell proliferation is an angiogenesis-related disease.

12. The method of Claim 4, wherein the angiogenic-related disease is a disease selected from the group consisting of cancer, arthritis, macular degeneration, and diabetic retinopathy.

13. A method of treating undesired angiogenesis in a human or animal comprising the steps of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of an angiogenesis-inhibiting compound comprising proteinases, proteinase inhibitors, serpins, proteinase/inhibitor complexes, thrombin/antithrombin, lipoproteins, and matrix proteins.

14. The method of Claim 13, wherein the angiogenesis-inhibiting compound is selected from the group consisting of alpha2-macroglobulin, beta-amyloid precursor protein, proteinase nexin I,
5 proteinase nexin II, pro-uPA, lipoprotein lipase, lactoferrin, PAI-1, horse leukocyte elastase inhibitor, protein C inhibitor, C1-inhibitor, alpha2-antiplasmin, alpha1-proteinase inhibitor, alpha1-antichymotrypsin, heparin cofactor II, tissue-type plasminogen activator, antithrombin III, tissue factor pathway inhibitor, apolipoprotein B, apolipoprotein J, clusterin,
10 thrombospondin, tissue factor pathway inhibitor 1, tissue factor pathway inhibitor 2, amyloid protein precursor, amyloid beta precursor protein, collagen VI, and bovine pancreatic trypsin inhibitor and active fragments thereof.

15. The method of Claim 14, further comprising a pharmaceutically acceptable excipient, carrier or sustained-release matrix.

16. The method of Claim 14, wherein the undesired angiogenesis is related to an angiogenic-related disease comprising cancer, arthritis, macular degeneration, and diabetic retinopathy.
20

17. A composition for treating undesired angiogenesis in a human or animal comprising an effective amount of an angiogenesis-inhibiting compound wherein the compound comprises ligands that bind
25 low density lipoprotein receptor.

18. The composition of Claim 17, wherein the low density lipoprotein receptor comprises low density lipoprotein, low density lipoprotein receptor-related protein, very low density lipoprotein receptor, gp330/megalin and active fragments thereof.
30

19. The composition of Claim 17, wherein the ligands comprise proteinases, proteinase inhibitors, serpins, proteinase/inhibitor complexes, thrombin/antithrombin, lipoproteins, matrix proteins and active fragments thereof.
35

20. The composition of Claim 17, wherein the ligand is selected from the group consisting of alpha2-macroglobulin, beta-amyloid precursor protein, proteinase nexin I, proteinase nexin II, pro-uPA, lipoprotein lipase, lactoferrin, PAI-1, horse leukocyte elastase inhibitor, protein C inhibitor, C1-inhibitor, alpha2-antiplasmin, alpha1-proteinase inhibitor, alpha1-antichymotrypsin, heparin cofactor II, tissue-type plasminogen activator, antithrombin III, tissue factor pathway inhibitor, apolipoprotein B, apolipoprotein J, clusterin, thrombospondin, tissue factor pathway inhibitor 1, tissue factor pathway inhibitor 2, amyloid protein precursor, amyloid beta precursor protein, collagen VI, bovine pancreatic trypsin inhibitor and active fragments thereof.

1/3

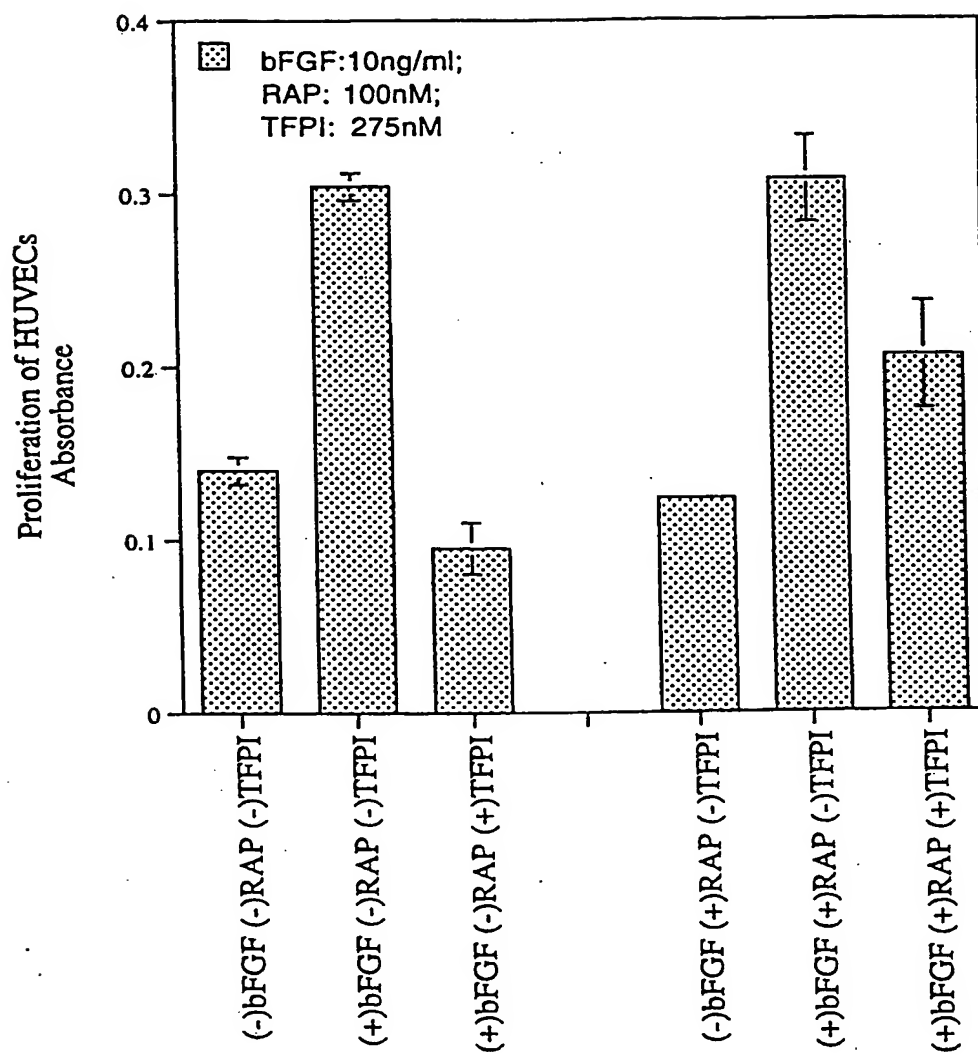


Figure 1

2/3

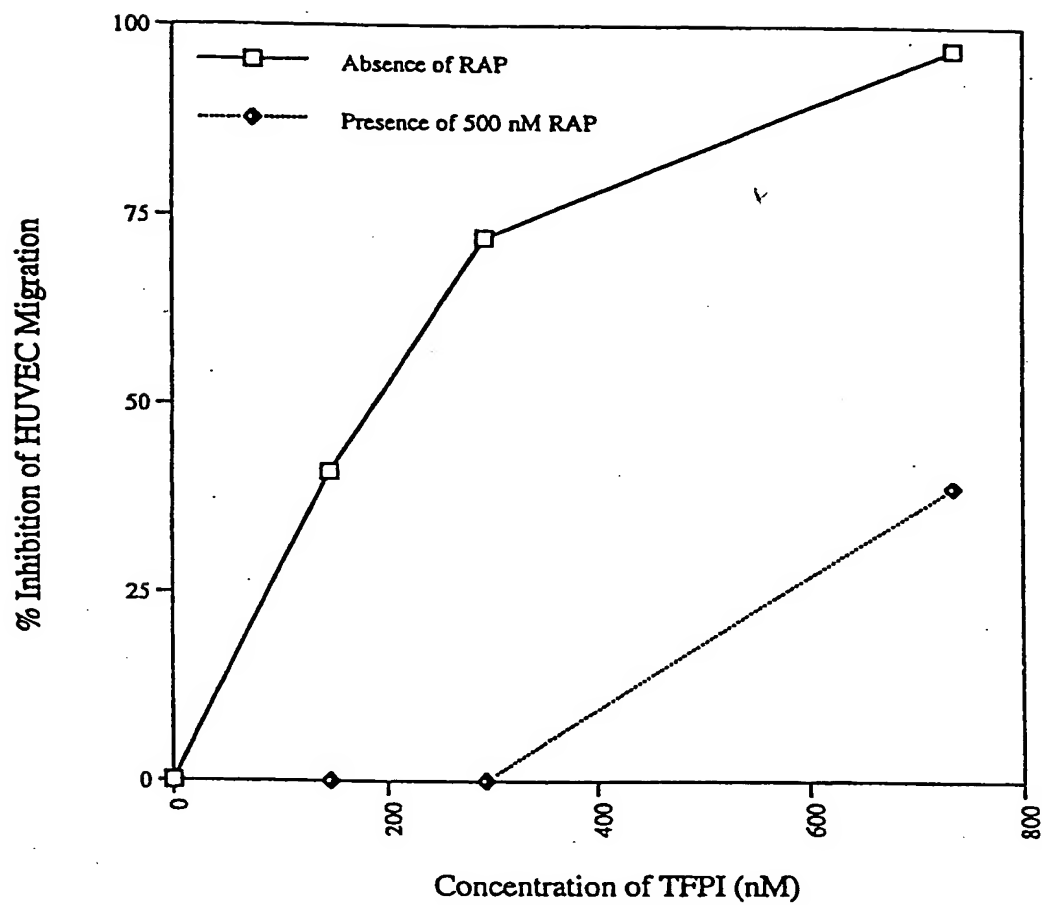


Figure 2

3/3

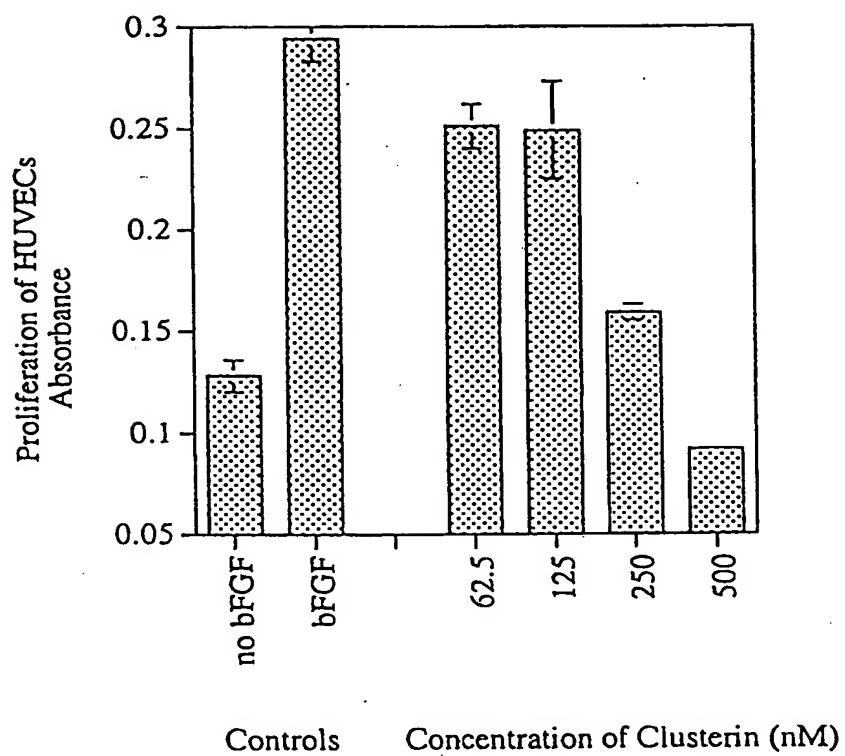


Figure 3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/07154

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/48 A61K38/57 A61K38/17 A61K38/39 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 98 34634 A (ENTREMED INC) 13 August 1998 (1998-08-13) abstract page 4, line 31 - page 5, line 35 page 9, line 19 - line 4 page 12, line 21 - line 27 page 13, line 23 - line 4 page 14, line 19 - line 33 claims 1-5, 10-16, 20; examples 1-4 --- -/-</p>	1-4, 8-20

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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- *Z* document member of the same patent family

Date of the actual completion of the international search

8 August 2000

Date of mailing of the international search report

23/08/2000

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Noë, V

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/07154

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 94 04178 A (BIO TECHNOLOGY GENERAL CORP) 3 March 1994 (1994-03-03)</p> <p>abstract page 18, line 29 -page 19, line 11; claims 1-11,19-23,27; examples 1-7 page 19, line 30 -page 20, line 19 page 3, line 33 -page 4, line 16 page 6, line 1 - line 34 page 14, line 1 - line 19 page 14, line 34 -page 15, line 1</p>	1-3, 8-11,13, 17-19
A	<p>HEMBROUGH W A ET AL: "Tissue factor pathway inhibitor (TFPI) is an inhibitor of angiogenesis." March 1999 (1999-03) , PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, VOL. 40, PAGE(S) 68 , 90TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH;PHILADELPHIA, PENNSYLVANIA, USA; APRIL 10-14, 1999, MARCH, 1999 XP002144496 ISSN: 0197-016X abstract</p>	1-20
A	<p>US 5 650 391 A (SCHWARTZ ALAN L ET AL) 22 July 1997 (1997-07-22) column 1, line 26 - line 28 column 2, line 3 - line 9 column 4, line 21 - line 29</p>	1-4
A	<p>KASZA ANETA ET AL: "Specificity of serine proteinase/serpin complex binding to very-low-density lipoprotein receptor and alpha-2-macroglobulin receptor/low-density-lipoprotein-receptor-related protein." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 248, no. 2, 1997, pages 270-281, XP000929788 ISSN: 0014-2956 abstract</p>	
A	<p>GOOD D J ET AL: "A TUMOR SUPPRESSOR-DEPENDENT INHIBITOR OF ANGIOGENESIS IS IMMUNOLOGICALLY AND FUNCTIONALLY INDISTINGUISHABLE FROM A FRAGMENT OF THROMBOSPONDIN" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 87, no. 17, 1990, pages 6624-6628, XP002144493 1990 ISSN: 0027-8424 abstract</p>	1-4,9,10
	<p>--- -/-</p>	

INTERNATIONAL SEARCH REPORT

In .ational Application No

PCT/US 00/07154

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WILLNOW THOMAS E ET AL: "Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 267, no. 36, 1992, pages 26172-26180, XP002144494 ISSN: 0021-9258 abstract page 26177, paragraph 2</p>	
A	<p>BAJOU K ET AL: "Absence of host plasminogen activator inhibitor 1 prevents cancer invasion and vascularization." NATURE MEDICINE, vol. 4, no. 8, August 1998 (1998-08), pages 923-928, XP002144495 ISSN: 1078-8956 page 923 page 924, column 2, paragraph 2</p>	

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Information on patent family members

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